

Claims

1. A method of designing a non-cytotoxic toxin conjugate for inhibition or reduction of exocytic fusion in a target cell, which method comprises:-

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(A) identifying an agonist that increases exocytic fusion in said target cell; and

(B) preparing an agent, which agent includes:-

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(i) a Targeting Moiety (TM) that binds the agent to a Binding Site on said target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist identifiable by step (A);

(ii) a non-cytotoxic protease or a fragment thereof, which protease or protease fragment is capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and

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(iii) a Translocation Domain that translocates the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

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2. A method of designing a non-cytotoxic toxin conjugate for inhibition or reduction of exocytic fusion in a target cell, which method comprises:-

(A) identifying an agonist that increases exocytic fusion in said target cell; and

(B) preparing an agent, which agent includes:-

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(i) a Targeting Moiety (TM) that binds the agent to a Binding Site on said target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist identifiable by step (A);

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(ii) a DNA sequence encoding a non-cytotoxic protease or a fragment thereof, which DNA sequence is expressible in the target cell and when so expressed provides a protease or protease fragment capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and

(iii) a Translocation Domain that translocates the DNA sequence encoding the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

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3. A method according to Claim 1 or Claim 2, comprising the step of confirming that the agonist increases exocytic fusion in the target cell.

4. A method according to any preceding claim, comprising detecting an increase in

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secretion from the target cell when agonist is present compared with when said agonist is absent.

5 5. A method according to Claim 4, wherein said detecting is performed by an assay employing chromatography, mass spectroscopy, and/or fluorescence.

6. A method according to Claim 4 or 5, wherein said detecting is performed by an assay employing ELISA/EIA/RIA techniques, and/or radio-tracer techniques.

10 7. A method according to any of Claims 1-3, comprising detecting an increase in the concentration of a cell membrane protein expressed at the cell surface of the target cell when agonist is present compared with when said agonist is absent.

15 8. A method according to Claim 7, wherein the cell membrane protein is a cell receptor protein, and the method comprises detecting an increase in the concentration of said receptor protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

20 9. A method according to Claim 7 or 8, wherein said detecting is performed by an assay employing immuno-histochemistry, flow cytometry, western blotting of isolated plasma membrane cell fractions, fluorescent-ligand binding techniques, and/or radio-ligand binding techniques.

25 10. A method according to Claim 7, wherein the cell membrane protein is a transporter protein, and the method comprises detecting an increase in the concentration of said transporter protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

30 11. A method according to Claim 7 or 10, wherein said detecting is performed by an assay employing immuno-histochemistry, flow cytometry, western blotting of isolated plasma membrane cell fractions, and/or intra- and extracellular assessment of transported material (eg. glucose).

35 12. A method according to Claim 7, wherein the cell membrane protein is a membrane channel protein, and the method comprises detecting an increase in the concentration of said membrane channel protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

13. A method according to Claim 7 or 12, wherein said detecting is performed by an assay employing biochemical assessment of ion concentration in an isolated sample (eg. serum, plasma, or urine), electrophysiology of tissue (eg. *ex vivo* tissue), intra- and extracellular assessment of transported material (eg. glucose), immuno-histochemistry, flow cytometry, and western blotting of isolated plasma membrane cell fractions.

14. A method of identifying an agonist that is suitable for re-targeting a non-cytotoxic protease or a fragment thereof to a target cell, which protease or fragment thereof is capable of cleaving a protein of the exocytic fusion apparatus in the target cell, said method comprising:-

- (A) identifying a putative agonist molecule;
- (B) contacting the target cell with said putative agonist molecule; and
- (C) confirming that said putative agonist molecule is an agonist by identifying an increase in exocytic fusion in the target cell when said molecule is present compared with when said molecule is absent.

15. A method according to Claim 14, comprising the step of confirming that the putative agonist molecule or agonist is capable of being combined with a non-cytotoxic protease (or a fragment thereof) or a DNA sequence encoding said protease (or the fragment thereof) to form an agent of the present invention.

16. A method according to Claim 14 or 15, comprising the step of confirming that said putative agonist molecule or agonist binds to a Binding Site on the target cell, which Binding Site is susceptible to receptor-mediated endocytosis.

17. A method according to any of Claims 14-16, comprising the step of confirming that said putative agonist molecule or agonist is able to deliver said non-cytotoxic protease (or fragment thereof), or a DNA sequence encoding said protease (or the fragment thereof), into the cytosol of a target cell.

18. A method according to any of Claims 14-17, wherein step (C) comprises detecting an increase in secretion from the target cell when agonist is present compared with when said agonist is absent.

19. A method according to Claim 18, wherein said detecting is performed by an assay employing chromatography, mass spectroscopy, and/or fluorescence.

20. A method according to Claim 18 or 19, wherein said detecting is performed by an assay employing ELISA/EIA/RIA techniques, and/or radio-tracer techniques.

5 21. A method according to any of Claims 14-17, wherein step (C) comprises detecting an increase in the concentration of a cell membrane protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

10 22. A method according to Claim 21, wherein the cell membrane protein is a cell receptor protein, and the method comprises detecting an increase in the concentration of said receptor protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

15 23. A method according to Claim 21 or 22, wherein said detecting is performed by an assay employing immuno-histochemistry, flow cytometry, western blotting of isolated plasma membrane cell fractions, fluorescent-ligand binding techniques, and/or radio-ligand binding techniques.

20 24. A method according to Claim 21, wherein the cell membrane protein is a transporter protein, and the method comprises detecting an increase in the concentration of said transporter protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

25 25. A method according to Claim 21 or 24, wherein said detecting is performed by an assay employing immuno-histochemistry, flow cytometry, western blotting of isolated plasma membrane cell fractions, and/or intra- and extracellular assessment of transported material (eg. glucose).

30 26. A method according to Claim 21, wherein the cell membrane protein is a membrane channel protein, and the method comprises detecting an increase in the concentration of said membrane channel protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

35 27. A method according to Claim 21 or 26, wherein said detecting is performed by an assay employing biochemical assessment of ion concentration in an isolated sample (eg. serum, plasma, or urine), electrophysiology of tissue (eg. *ex vivo* tissue), intra- and extracellular assessment of transported material (eg. glucose), immuno-histochemistry, flow cytometry, and western blotting of isolated plasma membrane cell fractions.

28. A method according to any preceding claim, wherein the protease is a bacterial protein, or a fragment thereof capable of cleaving a protein of the exocytic fusion apparatus of the target cell.

5 29. A method according to Claim 28, wherein the bacterial protein is selected from a clostridial neurotoxin, or an IgA protease.

30. A pharmaceutical composition, which includes an agent comprising:-

- 10 (i) a Targeting Moiety (TM) that binds the agent to a Binding Site on a target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist that is capable of increasing exocytic fusion in the target cell;
- (ii) a non-cytotoxic protease or a fragment thereof, which protease or protease fragment is capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and
- 15 (iv) a Translocation Domain that translocates the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

20 31. A pharmaceutical composition, which includes an agent comprising:-

- (i) a Targeting Moiety (TM) that binds the agent to a Binding Site on a target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist that is capable of increasing exocytic fusion in the target cell;
- 25 (ii) a DNA sequence encoding a non-cytotoxic protease or a fragment thereof, which DNA sequence is expressible in the target cell and when so expressed provides a protease or protease fragment capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and
- (iii) a Translocation Domain that translocates the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.
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32. A composition according to Claim 30 or 31, wherein the agonist is capable of contacting the target cell and increasing secretion from said target cell compared with when the agonist is absent.

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33. A composition according to Claim 30 or 31, wherein the agonist is capable of contacting the target cell and increasing the concentration of a cell membrane protein expressed at

the cell surface of said target cell compared with when the agonist is absent.

5 34. A composition according to Claim 33, wherein the agonist is capable of contacting the target cell and increasing the concentration of a cell receptor protein expressed at the cell surface of said target cell compared with when the agonist is absent.

10 35. A composition according to Claim 33, wherein the agonist is capable of contacting the target cell and increasing the concentration of a transporter protein expressed at the surface of said target cell compared with when the agonist is absent.

36. A composition according to Claim 33, wherein the agonist is capable of contacting the target cell and increasing the concentration of a membrane channel protein expressed at the surface of said target cell compared with when the agonist is absent.

15 37. A composition according to any of Claims 30-36, wherein said agent has been prepared by a method according to any of Claims 1-13.

38. A composition according to any of Claims 30-36, wherein said agonist has been identified by a method according to any of Claims 14-27.

20 39. A composition according to any of Claims 30-38, further comprising an inhibitor that alleviates, in a patient, clinical symptoms caused by exocytic fusion in said target cell.

25 40. A composition according to Claim 39, wherein the inhibitor alleviates the clinical symptoms caused by increased exocytic fusion resulting from binding of the agonist to the target cell.

30 41. A composition according to Claim 39 or 40, wherein the inhibitor has a short-acting duration once administered to a patient, preferably a short-acting duration of 1-3 days, more preferably a short-acting duration of 1-2 days, most preferably a short-acting duration of 24-36 hours.

35 42. A composition according to any of Claims 30-41, wherein the protease is a bacterial protein, or a fragment thereof capable of cleaving a protein of the exocytic fusion apparatus of the target cell.

43. A composition according to Claim 42, wherein the bacterial protein is selected from a clostridial neurotoxin, or an IgA protease.

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44. A DNA construct encoding the agent defined in Claim 30, said construct comprising a DNA encoding the TM and/or the Translocation Domain, and the protease (or fragment thereof).

5 45. A method of preparing the agent defined in Claim 30, comprising expressing the DNA construct of Claim 44 in a host cell.

46. A method of preparing the agent defined in Claim 30, comprising covalently linking the TM and/or Translocation Domain, and the protease (or fragment thereof).

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47. A method of preparing the agent defined in Claim 31, comprising covalently linking the TM and/or Translocation Domain, and the DNA sequence encoding the protease (or the fragment thereof).

15 48. Use of a composition according to any of Claims 30-43 for the manufacture of a medicament for treating a medical disease or condition in a patient, wherein the disease or condition is caused by exocytic fusion in a target cell of said patient

20 49. Use of a composition according to any of Claims 30-38 for the manufacture of a medicament for treating a medical disease or condition in a patient, wherein the disease or condition is caused by exocytic fusion in a target cell of said patient.

25 50. Use of a composition according to Claim 49, wherein the medicament is to be administered to the patient prior to, simultaneously with, or subsequent to an inhibitor, and wherein the inhibitor alleviates, in the patient, clinical symptoms caused by exocytic fusion.

51. Use of a composition according to Claim 50, wherein the inhibitor alleviates, in the patient, clinical symptoms caused by increased exocytic fusion resulting from binding of the agonist to the target cell.

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52. Use of a composition according to Claim 50 or 51, wherein the inhibitor has a short-acting duration once administered to the patient, preferably a short-acting duration of 1-3 days, more preferably a short-acting duration of 1-2 days, most preferably a short-acting duration of 24-36 hours.

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53. A method for treating a medical disease or condition caused by exocytic fusion in a target cell, comprising administering to a patient a composition according to any of Claims 30-43.

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54. A method for treating a medical disease or condition caused by exocytic fusion in a target cell, comprising administering to a patient a composition according to any of Claims 30-38.
- 5 55. A method according to Claim 54, wherein the composition is administered to a patient prior to, simultaneously with, or subsequent to an inhibitor, wherein the inhibitor alleviates, in the patient, clinical symptoms caused by exocytic fusion.
- 10 56. A method according to Claim 55, wherein the inhibitor alleviates, in the patient, clinical symptoms caused by increased exocytic fusion resulting from binding of the agonist to the target cell.
- 15 57. A method according to Claim 55 or 56, wherein the inhibitor has a short-acting duration once administered to the patient, preferably a short-acting duration of 1-3 days, more preferably a short-acting duration of 1-2 days, most preferably a short-acting duration of 24-36 hours.